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Isolation and characterization of the human $G_s\alpha$ gene

(GTP-binding protein/alternative splicing/promoter/adenylate cyclase)

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ABSTRACT **The gene for $G_s\alpha$ (the α subunit of the guanine nucleotide-binding protein G_s) was isolated from human genomic libraries using rat $G_s\alpha$ cDNA as a probe. Comparison of the nucleotide sequence of the human gene with that of the rat cDNA revealed that the human $G_s\alpha$ gene spans ≈20 kilobases and is composed of 13 exons and 12 introns. Genomic Southern blot analysis suggests that the human haploid genome contains a single $G_s\alpha$ gene. Previous reports indicated the presence of multiple species of $G_s\alpha$ cDNA. The structure of the human $G_s\alpha$ gene suggests that four types of $G_s\alpha$ mRNAs may be generated from a single $G_s\alpha$ gene by alternate use of exon 3 and/or of two 3′ splice sites of intron 3, where an unusual splice junction sequence (TG) instead of the consensus (AG) is used. S1 nuclease mapping analysis of human $G_s\alpha$ mRNA identified multiple transcriptional initiation sites. The promoter region of the human $G_s\alpha$ gene has extremely high G+C content (85%). It contains 4 "GC" boxes, but no typical "TATA" or "CAAT" box sequence. In the 5′ flanking region, there are several blocks of sequences that are similar to the sequences of the 5′ flanking region of the human c-Ki-*ras2* gene.**

G proteins are a family of guanine-nucleotide binding proteins that are involved in various transmembrane signaling systems (1). G_s activates and G_i inhibits adenylate cyclase in response to hormonal stimuli (1), whereas transducin (G_t) regulates cGMP phosphodiesterase activity in visual transduction (2). G_o is another G protein that is present predominantly in brain tissues, although its precise function has not yet been clarified (3).

G proteins are heterotrimers composed of α, β, and γ subunits. The α subunits of G protein ($G\alpha$) bind guanine nucleotide and are unique to each G protein. Recent progress in molecular cloning of $G\alpha$ cDNAs revealed that they are highly similar proteins (4). So far, four $G_s\alpha$ (5), three $G_i\alpha$ (4, 6, 41), two $G_t\alpha$ (7), and $G_o\alpha$ (4) cDNAs were isolated and their sequences were determined (see also ref. 1). There may be still some other species of $G\alpha$ cDNAs, since the coupling of G proteins with other signal transduction systems such as activation of phospholipase C (8) and phospholipase A_2 (9) or gating of ion channels (10) has been suggested.

The presence of two species of $G_s\alpha$ protein with different molecular masses (45 and 52 kDa) was known (11). Recently, Bray *et al.* (5) isolated four different $G_s\alpha$ cDNAs ($G_s\alpha$-1 to -4) from human brain and characterized the partial structure. $G_s\alpha$-1 and $G_s\alpha$-3 are identical except that $G_s\alpha$-3 lacks a single stretch of 45 nucleotides. $G_s\alpha$-2 and $G_s\alpha$-4 have 3 additional nucleotides (CAG) to $G_s\alpha$-1 and $G_s\alpha$-3 3′ to the above 45 nucleotides. Robishaw *et al.* (12) isolated two $G_s\alpha$ cDNAs from bovine adrenal that correspond to $G_s\alpha$-1 and $G_s\alpha$-4. They showed that these two cDNAs generated a 52- and a 45-kDa protein when expressed in COS-m6 cells. Mattera *et al.* (13) also isolated two $G_s\alpha$ cDNAs from human liver that correspond to $G_s\alpha$-1 and $G_s\alpha$-4.

In this report, we isolated and characterized the human $G_s\alpha$ chromosomal gene.* The human $G_s\alpha$ gene is a split gene, having 13 exons and 12 introns that span ≈20 kilobases (kb) of genomic DNA. From the exon–intron organization, it is suggested that four types of mRNAs found by Bray *et al.* (5) may be derived from a single $G_s\alpha$ gene by alternative splicing of mRNA precursors.

MATERIALS AND METHODS

Isolation of Genomic Clones of Human $G_s\alpha$. The human genomic libraries constructed from human fetal liver (14) and human placenta (15) were kindly provided by T. Maniatis (Harvard University) and M. Shibuya (University of Tokyo), respectively. The rat $G_s\alpha$ cDNA (4) was labeled with ^{32}P by nick-translation (16) or the random-primer method (17). About 3×10^5 or 7×10^5 plaques of the human genomic library were screened by plaque hybridization (18) with ^{32}P-labeled rat $G_s\alpha$ cDNA. Hybridization was carried out at 42°C in 50% formamide/5× SSC (1× SSC = 0.15 M NaCl/15 mM sodium citrate)/1× Denhardt's solution (0.02% bovine serum albumin/0.02% polyvinylpyrrolidone/0.02% Ficoll)/20 mM sodium phosphate, pH 7.0/100 μg of heat-denatured calf thymus DNA per ml/0.1% $NaDodSO_4$/10% dextran sulfate. Filters were washed twice at room temperature in 6× SSC/0.1% $NaDodSO_4$ for 15 min and then with 0.1× SSC/0.1% $NaDodSO_4$ for 30 min before autoradiography.

DNA Sequence Analysis. The nucleotide sequence was determined by using phage M13 vectors and the dideoxy chain-termination method (19, 20).

Genomic Southern Blot Analysis. High molecular weight genomic DNA was extracted from human peripheral leukocytes. DNA was digested with *Hin*dIII or *Bgl* II, and the fragments were electrophoresed on a 0.7% agarose gel and transferred to nitrocellulose filters. Hybridization was carried out with ^{32}P-labeled human $G_s\alpha$ genomic fragments as described above. Filters were washed at 65°C twice with 6× SSC/0.1% $NaDodSO_4$ for 15 min and then with 0.1× SSC/0.1% $NaDodSO_4$ for 1 hr before autoradiography.

S1 Nuclease Mapping Analysis. Total cellular RNA was extracted from human promyelocytic leukemia HL60 cells by the guanidium thiocyanate/cesium chloride method as described by Chirgwin *et al.* (21). Poly(A)$^+$ RNA was selected by oligo(dT)-cellulose chromatography. The 1.3-kb *Eco*RI/*Pvu* II or 0.8-kb *Nco* I/*Xho* I fragment, which covers the 5′-flanking region of the $G_s\alpha$ gene, was labeled at the 5′ end with [γ-^{32}P]ATP and T4 polynucleotide kinase (8×10^5

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Abbreviations: G proteins, guanine nucleotide-binding regulatory proteins; G_s and G_i, G proteins that mediate stimulation and inhibition, respectively, of adenylate cyclase; G_t, transducin; G_o, a G protein of unknown function; $G_s\alpha$, $G_i\alpha$, $G_t\alpha$, and $G_o\alpha$, α subunits of G_s, G_i, G_t, and G_o, respectively.

*This sequence is being deposited in the EMBL/GenBank data base (Bolt, Beranek, and Newman Laboratories, Cambridge, MA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03647).

cpm per pmol of DNA). About 0.05 pmol of each probe was denatured at 90°C for 3 min and hybridized at 60°C for 16 hr with 10 μg of poly(A)$^+$ RNA from HL60 cells and treated with S1 nuclease (800 units/ml) (Pharmacia) as described (22). The products were analyzed by electrophoresis on a 5% polyacrylamide gel containing 7 M urea and detected by autoradiography.

RESULTS

Isolation and Characterization of the Human $G_s\alpha$ Gene. About 3×10^5 plaques of human genomic library (14) were screened with ^{32}P-labeled rat $G_s\alpha$ as a probe (4). Five clones were isolated and all showed the same restriction map. Restriction fragments from one of the clones, λHGS51, which hybridized with the rat $G_s\alpha$ probe, were subcloned into pBR vectors. Comparison of the nucleotide sequence with that of the rat $G_s\alpha$ cDNA revealed that the clone λHGS51 contained exons 7–13 of the $G_s\alpha$ gene but lacked the further 5′ part of the gene. Therefore, we have rescreened 7×10^5 plaques of another human genomic library (15) and isolated two clones, λHGS31 and λHGS33. Nucleotide sequence analysis revealed that three clones overlap as shown in Fig. 1 and that λHGS31 and λHGS33 covered the entire $G_s\alpha$ gene.

The human $G_s\alpha$ gene is composed of 13 exons and 12 introns (Fig. 1). All of the splice junction sequences conformed to the GT–AG rule for the nucleotide immediately flanking the exon borders (Fig. 2) (23). In the coding region, the nucleotide sequence similarity between rat and human $G_s\alpha$ was 95% and the similarity of the deduced amino acid sequence was >99%. There was only a single amino acid replacement, Asn-139 in rat being changed to Asp-139 in human $G_s\alpha$. Similarities between rat and human of the 5′ and 3′ flanking regions were ≈90%, although the 5′ flanking region of the human $G_s\alpha$ gene contained an insertion sequence of ≈100 base pairs (from −75 to −185 in Fig. 2) when compared with the rat cDNA. No splicing signals were found around this sequence. There were 6 polyadenylylation signals (AATAAA) in the 3′ flanking region. The nucleotide sequence of the human chromosomal gene and that of human liver cDNA (13) were identical from −48 to +1546 [numbers refer to the sequences in figure 2 of Mattera *et al.* (13)] except for 1 nucleotide; the C at position 393 in their cDNA sequence is a T in our chromosomal sequence. This difference did not change the amino acid Ile-131.

Fig. 3 shows genomic Southern blot analysis of the $G_s\alpha$ gene. The 0.5-kb *Nco* I/*Nco* I fragment and the 0.4-kb *Nco* I/*Ava* I fragment, which contain the coding region of exon 1 and exon 13, respectively, were used as probes. A single band was detected in the *Hin*dIII or *Bgl* II digests of chromosomal DNA with both probes (Fig. 3). A similar experiment carried out with the *Bam*HI digests revealed single bands of 12 and 2 kb with the 5′ and 3′ probes, respectively (data not shown). From these results, it was concluded that the human haploid genome contains only one $G_s\alpha$ gene.

Alternative Splicing of $G_s\alpha$ mRNAs. Comparison of the four types of human $G_s\alpha$ cDNAs reported by Bray *et al.* (5) with the sequence of the human $G_s\alpha$ gene suggests that four types of $G_s\alpha$ mRNAs may be generated from a single $G_s\alpha$ gene by alternative splicing as shown in Fig. 4. $G_s\alpha$-1 has a sequence identical to exons 2, 3, and 4, whereas $G_s\alpha$-3 lacks a stretch of 45 nucleotides of $G_s\alpha$-1, which coincides with exon 3. Therefore, $G_s\alpha$-1 and $G_s\alpha$-3 may be derived by the alternative use of exon 3. $G_s\alpha$-2 has 3 additional nucleotides (CAG) to $G_s\alpha$-1 at the 3′ end of the above 45 nucleotides. $G_s\alpha$-4 also has these 3 additional nucleotides to $G_s\alpha$-3 between exons 2 and 4. In the genomic sequence of the 3′ splice site of intron 3, this CAG sequence is found. The 5′ adjacent nucleotides to the CAG are TG and do not match with the 3′ splice consensus sequence AG. However, upstream of the splice site is the pyrimidine-rich sequence, which agrees with the rest of the 3′ splice consensus sequence (23). This 3′ splice site may be used for the production of $G_s\alpha$-2 and $G_s\alpha$-4.

Characterization of the 5′ Flanking Region of the $G_s\alpha$ Gene. The nucleotide sequence upstream of the initiation codon ATG is shown in Fig. 2. The G+C content of this region is extremely high (85%). To determine the transcriptional initiation site of the human $G_s\alpha$ gene, S1 nuclease mapping analyses were performed. Ten micrograms of poly(A)$^+$ RNA from HL60 cells was hybridized at 60°C with the ^{32}P-labeled 1.3-kb *Eco*RI/*Pvu* II fragment or the 0.8-kb *Nco* I/*Xho* I fragment of the 5′ end of the $G_s\alpha$ gene as shown in Fig. 5. In lane 1, only one band of ≈400 nucleotides was detected with the *Eco*RI/*Pvu* II fragment as a probe. To determine the initiation site more precisely, we used the *Nco* I/*Xho* I fragment as a probe. As shown in lane 3, one band of 106 nucleotides and several bands of 96–101 nucleotides were detected. From the sizes of these protected bands, it was concluded that there are two or more transcriptional initiation sites in the $G_s\alpha$ gene (between −338 and −328 in Fig. 2).

No typical "TATA" box or "CAAT" box was found in the promoter region of the $G_s\alpha$ gene. The "GC" box (GGGCGG or CCGCCC) is the possible binding site for the transcriptional factor Sp1, which activates the simian virus 40 early promoter (24). Recent work indicates that it can also bind to GC boxes and stimulate the transcription of several viral and cellular genes (25). In the $G_s\alpha$ gene, the GGGCGG sequence is repeated twice at positions −552 and −363, and the CCGCCC sequence is repeated twice at positions −497 and −389. A sequence of ≈250 base pairs in intron 1 also had a high G+C content (85%), and seven GC box sequences were found in this region, as shown in Fig. 2.

Another repeated sequence TCCTCCTCC was found three times in the promoter region at −453, −444, and −410. The same sequence was found four times in the promoter region of the human epidermal growth factor receptor gene (26) and two of its inverted complement GGAGGAGGA were found in the promoter region of the human c-*erbB2* gene (27).

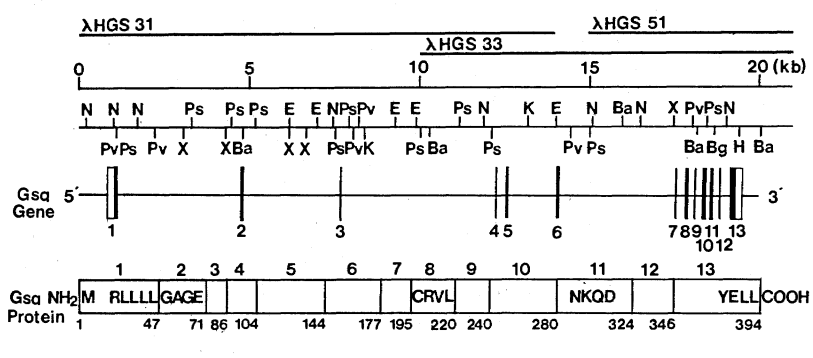

FIG. 1. Organization of the human $G_s\alpha$ gene. The top heavy lines represent the DNA inserts contained within the genomic clones. Size scale and location of the major restriction enzyme recognition sites are shown below. The 13 exons and 12 introns of the $G_s\alpha$ gene are represented by boxes and lines. The coding or the noncoding sequence is shown by a solid box or an open box, respectively. $G_s\alpha$ protein is represented by the lowest bar and is divided by vertical lines for each exon. Distinctive amino acid sequences of $G_s\alpha$ protein are shown by one-letter abbreviations. The number of amino acids at the end of each exon is indicated below. Ba, *Bam*HI; Bg, *Bgl* II; E, *Eco*RI; H, *Hin*dIII; K, *Kpn* I; N, *Nco* I; Ps, *Pst* I; Pv, *Pvu* II; X, *Xba* I.

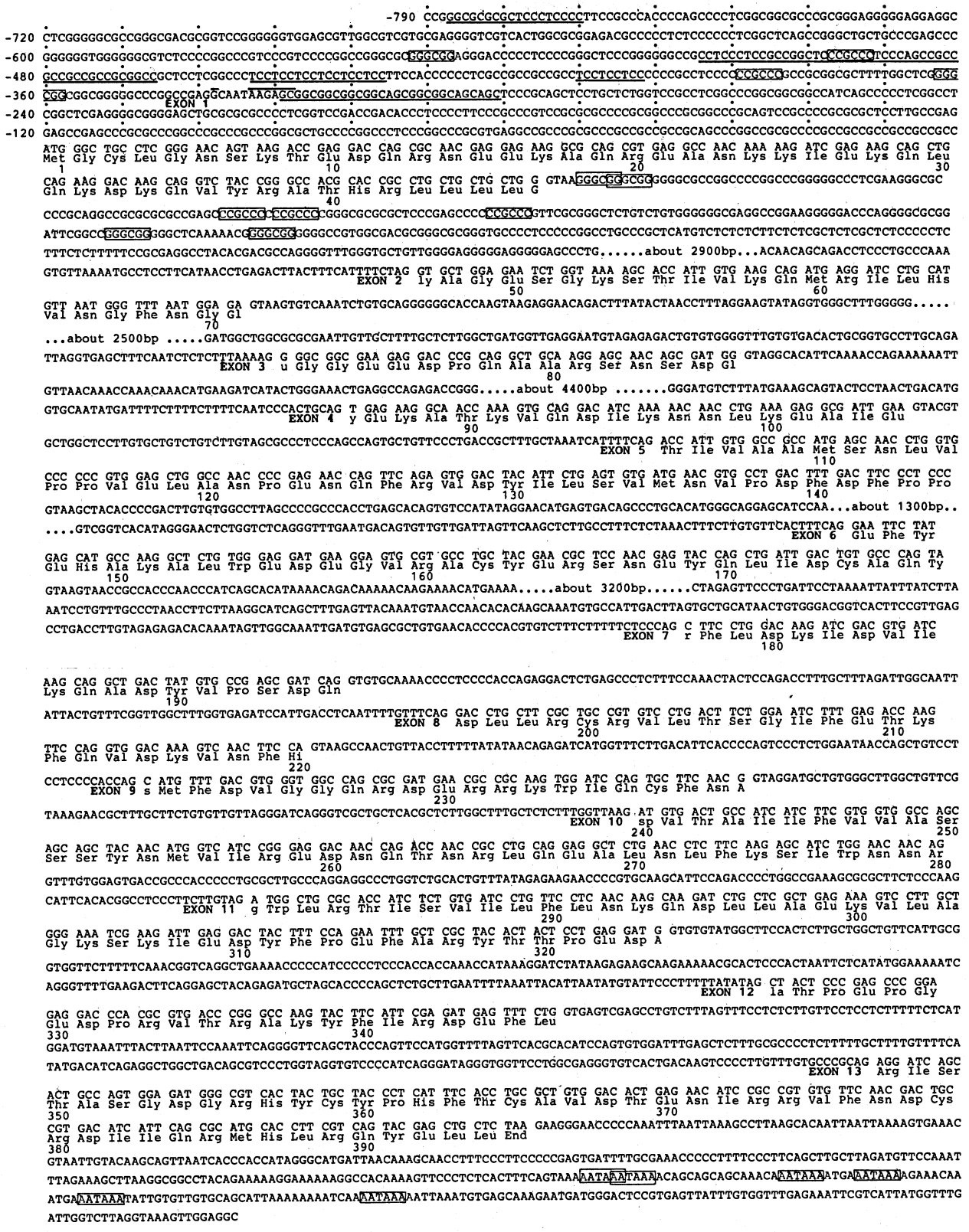

FIG. 2. Nucleotide sequence of the human $G_s\alpha$ gene. The coding sequences of the exons are translated and amino acid numbers from the ATG initiation codon are shown. The GC box sequences in the promoter region and intron 1 are boxed. The repeated sequences TCCTCCTCC and the three similar sequences with the 5′ flanking region of the human c-Ki-*ras2* gene are underlined. The transcriptional initiation sites determined by S1 nuclease mapping analysis are overlined. On the 3′ flanking region, AATAAA polyadenylylation signals are boxed.

DISCUSSION

The human chromosomal gene of $G_s\alpha$ is a split gene composed of 13 exons and 12 introns extending ≈20 kb. We have recently isolated and characterized the chromosomal genes for human $G_i2\alpha$ and $G_i3\alpha$ (41), both of which are composed of 8 exons and 7 introns in the coding region. The alignment of their exons indicates that they possess completely identical exon junctions. The comparison of gene organization of the human $G_s\alpha$ and $G_i\alpha$ genes revealed that they also share

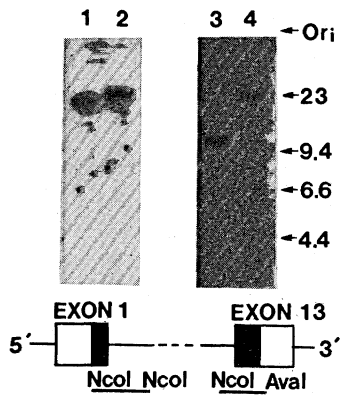

FIG. 3. Genomic Southern blot analysis of the human $G_s\alpha$ gene. Ten micrograms of human genomic DNA was digested with *Bgl* II (lanes 1 and 3) or *Hin*dIII (lanes 2 and 4) and electrophoresed on a 0.7% agarose gel. DNA was transferred to a nitrocellulose filter and hybridized as described. The probe was a 0.5-kb *Nco* I/*Nco* I fragment, which contains the coding region of exon 1 (lanes 1 and 2), or a 0.4-kb *Nco* I/*Ava* I fragment, which contains the coding region of exon 13 (lanes 3 and 4) as shown below the autoradiograms. *Hin*dIII-digested λ DNA was used as a size marker and the sizes of the DNA fragments are given in kb. Ori, origin of electrophoresis.

three identical exon junctions: junctions of exons 1 and 2, 6 and 7, and 8 and 9 of $G_s\alpha$ corresponding to junctions of exons 1 and 2, 4 and 5, and 5 and 6 of $G_i\alpha$, respectively. These three junctions are in the highly conserved regions among all G_αs. This suggests that α subunits of signal-transducing G proteins evolved from a common ancestral gene and that $G_s\alpha$ probably diverged from the $G_i\alpha$ family in an early stage of evolution.

The exon–intron organization of the $G_s\alpha$ gene was compared with the predicted functional domain structure of $G_s\alpha$ protein (28). The region that is believed to be responsible for GTPase activity is encoded in exons 1 and 2. Exon 3 is unique to $G_s\alpha$ and exons 4 and 5 encode the region that is heterogeneous among G_αs. This region may be the interaction site for an amplifier molecule, adenylate cyclase in the case of $G_s\alpha$. Exons 7–11 encode the region that is conserved among all G protein α subunits. Exon 8 contains Arg-201, which is ADP-ribosylated in the presence of cholera toxin (29). The conserved Asp-223 in exon 9 may form a salt bridge to Mg^{2+}, which is linked to the β phosphoryl group of GDP (30). The exchange of GDP to GTP may result in displacement of the surrounding region (residues 221–230) and thereby produce GTP-dependent conformational change in the following hydrophilic region (residues 230–238) in exon 9. A nonhydrolyzable GTP analog, but not GDP, prevents tryptic cleavage at Lys-233 in G_t or G_o (31). Exon 11 contains the consensus sequence Asn-Lys-Xaa-Asp in amino acid residues 292–295. In elongation factor Tu, earlier biochemical studies indicated that this region is responsible for interaction with guanine nucleotide (see ref. 32), and later

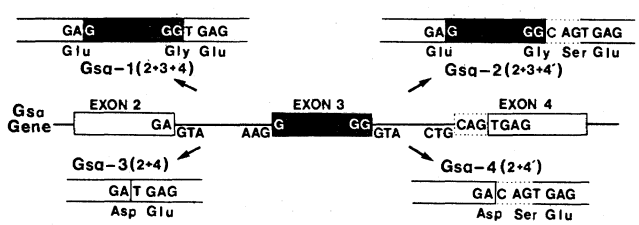

FIG. 4. Model for origin of four different $G_s\alpha$ mRNAs by alternative splicing. The $G_s\alpha$ gene is shown in the center. Exons 2 and 4 are shown by open boxes, exon 3 is shown by a solid box. Nucleotide sequences of exon–intron boundaries are shown. Four $G_s\alpha$ mRNAs are indicated by $G_s\alpha$-1, -2, -3, and -4.

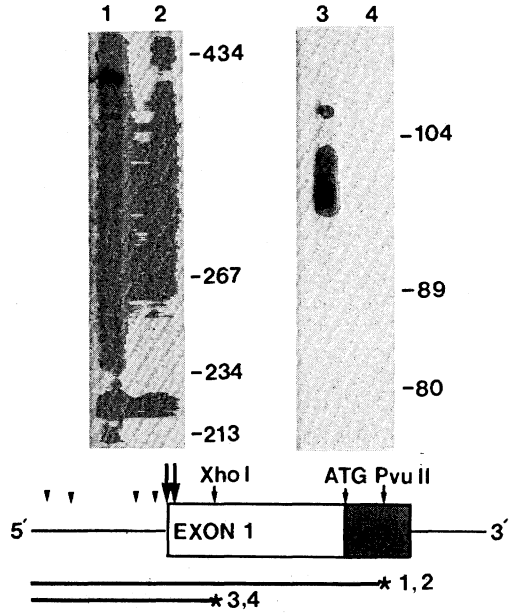

FIG. 5. S1 nuclease mapping analysis of human $G_s\alpha$ mRNA. The ^{32}P 5'-end-labeled *Eco*RI/*Pvu* II fragment (lanes 1 and 2) or *Nco* I/*Xho* I fragment (lanes 3 and 4) of the 5' end of the $G_s\alpha$ gene was annealed with (lanes 1 and 3) or without (lanes 2 and 4) 10 μg of poly(A)$^+$ RNA from HL60 cells and treated with S1 nuclease. The products were analyzed by 5% polyacrylamide gel containing 7 M urea and detected by autoradiography. The numbers on the right of the autoradiograms are the sizes of ^{32}P-labeled *Hae* III-digested pBR322 markers. Below the autoradiograms, the 5' end of the human $G_s\alpha$ gene is shown. The transcriptional initiation sites corresponding to S1 nuclease-resistant products are indicated by large arrows. Four GC boxes are indicated by arrowheads above the gene. At the bottom, the 5'-end-labeled (asterisk) probes are shown.

the four residues Asn-Lys-Cys-Asp are found to be situated close to the guanine ring by x-ray analysis (30).

In the case of $G_i\alpha$, pertussis toxin ADP-ribosylates a cysteine residue located at the fourth position from the COOH terminus and uncouples G_i from the receptor. In $G_s\alpha$, tyrosine instead of cysteine is present in this position and pertussis toxin does not affect the function of G_s. Exon 13 and possibly 12 may encode a domain of $G_s\alpha$ protein that interacts with its receptor.

An alternative splicing model proposed in this paper to explain the generation of four different $G_s\alpha$ mRNAs implies the use of an unusual 3' splice site sequence of TG. In most of the cases, the GT–AG rule for splicing is strictly conserved (23). However, there are some exceptional cases in which GC instead of GT is used for the 5' splice site (33, 34). Recently, in the *Drosophila* clock gene, the unusual 3' splice site CG is reported (35). Since genomic Southern blot analysis indicates the presence of only a single $G_s\alpha$ gene per haploid genome, we consider that this unusual sequence TG at the 3' splice site may probably be used. The amino acid sequence of exon 3, which is deleted in the differential splicing, is hydrophilic and is present on the surface of the $G_s\alpha$ protein. It may have some functional role in the adenylate cyclase system. One additional serine residue in $G_s\alpha$-2 and $G_s\alpha$-4 may be the potential site for phosphorylation by protein kinase C (36). Thus, the alternative use of these splice sites may confer $G_s\alpha$ proteins with differential regulatory properties.

The promoter region of the $G_s\alpha$ gene has a high G+C content and 4 GC boxes. It has neither a typical TATA box nor a CAAT box. S1 nuclease mapping analysis shows multiple transcriptional initiation sites. Promoter regions of other housekeeping genes such as the human epidermal

Table 1. Sequence identity in the 5′ flanking regions of the human $G_s\alpha$ gene and the human c-Ki-*ras2* gene

Sequence 1	−787	GGCGCGCGCTCCCTCCCC	−770
	240	GGCGCTCGCTGCCTCCCC	257
Sequence 2	−514	CCTCCCTCCGCCGGCTCCCGCCCTCCCAGCCGCCGCCGCCGCCGCGGCC	−466
	171	CCTTCCTCCGCCGGC-CCGGCCCCCGCT-CCTCCCCCGCCGGCCCGGCC	217
Sequence 3	−329	GCGGCGGCGGCGGCAGCGGCGGCAGCAGC	−301
	405	GCGGCGGCCGCGGCGGCGGAGGCAGCAGC	433

The upper and lower lines show the sequences from the 5′ flanking regions of the human $G_s\alpha$ gene (Fig. 2) and the human c-Ki-*ras2* gene (39), respectively. The numbers of the $G_s\alpha$ gene indicate the number of nucleotides from the initiation codon ATG. The numbers of the c-Ki-*ras2* gene refer to the sequence in figure 3 of ref. 39.

growth factor receptor gene (26), the human c-Ha-*ras* (*HRAS*) gene (37), and the human adenosine deaminase gene (38) have similar features. They are G+C-rich and contain multiple GC boxes. They often lack typical TATA or CAAT boxes. Multiple transcriptional initiation sites are usually present. These genes are expressed in a variety of tissues. In rat, $G_s\alpha$ was expressed in all tissues so far examined (data not shown). Expression of the $G_s\alpha$ gene may be controlled by the same mechanism as these housekeeping genes.

By computer analysis, a significant homology was detected between the 5′ flanking region of the human $G_s\alpha$ gene and the human c-Ki-*ras2* gene (39). Three blocks of similar sequences are shown in Table 1, of which the similarity of sequence 3 is most striking, with 26 of 29 nucleotides being identical. In the $G_s\alpha$ gene, sequence 3 is found immediately downstream of the transcriptional initiation site. In the c-Ki-*ras2* gene, sequence 3 is found near the transcriptional initiation site (40), and furthermore, sequences 1 and 2 are found in the region that is hypersensitive to DNase I and micrococcal nuclease (40). A similar sequence was also found between the 5′ flanking region of the human $G_i2\alpha$ gene and the human c-Ha-*ras* gene (41). These similar sequences may have some significant roles in the transcriptional control of the c-Ki-*ras2* gene and the $G_s\alpha$ gene or of the c-Ha-*ras* gene and the $G_i2\alpha$ gene.

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